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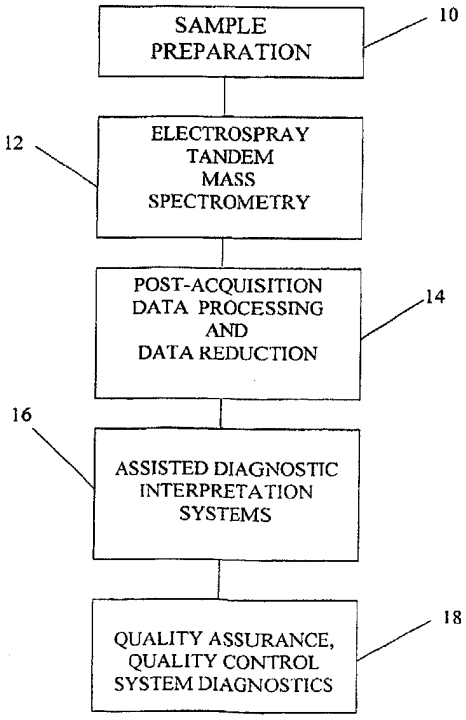
(54) Title: CLINICAL METHOD FOR THE GENETIC SCREENING OF NEWBORNS USING TANDEM MASS SPECTROMETRY



(57) Abstract: A high-throughput method for screening newborns using electrospray tandem mass spectrometry (12). The method improves the current protocols using tandem mass spectrometry to provide accurate and consistent results at the clinical level through enhanced quality assurance and quality control (18) protocols for scan profiling and sample preparation (10) of blood from newborns. Precise concentrations of specific internal standards are used to to distinguish twenty metabolites. Spectra of the samples are scanned and vigorously compared to known spectra as part of a diagnostic interpretation system (16). Quality assurance flags step compare peaks, metabolite concentration and scan intensities to a range of thresholds to determine whether or not the sample is contaminated, drug-ridden, diagnosable or unacceptable. All results and quality assurance flags are organized for post-acquisition data processing and data reduction (14) where values are compiled and stored for daily output results and trend analysis.

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— *with amended claims*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF INVENTION:

CLINICAL METHOD FOR THE GENETIC SCREENING OF NEWBORNS USING TANDEM MASS SPECTROMETRY

TECHNICAL FIELD:

Genetically screening newborn babies using tandem mass spectrometry. In particular, blood spots taken from newborns are combined with internal standards and scanned to quantify the plurality of blood metabolites to assist in the diagnosis of metabolic disorders. Quality controls and quality assurance indicators used within the internal standards and the scan functions assure accurate sampling techniques and analysis for assistance in medical diagnosis.

BACKGROUND ART:

Mass spectrometry has been making significant contributions to the diagnosis of metabolic diseases for over 20 years. Fast Atom Bombardment Tandem Mass Spectrometry (FAB-MS/MS) analysis of acylcarnitines in very small volumes of whole blood or plasma has been previously made routine. See Millington, et al., *Mass Spectrometry: Clinical and Biomedical Applications*, 1, ch. 8, 299-318. It had been a very satisfactory biochemical method for the differential diagnosis of disorders of fatty acid catabolism, and the instrumental method recognized numerous defects of branched-chain amino acid catabolism. The frequency of occurrence of these diseases and their association with sudden, unexplained deaths has generated a great medical interest in the development of neonatal screening tests.

Routine analysis of amino acids and acylcarnitines by Liquid Secondary Ion Tandem Mass Spectrometry (LSIMS/MS) from blood spots on filter paper has been demonstrated previously as well. See Chace et al., "Neonatal Screening for Inborn Errors of Metabolism by Automated Dynamic Liquid Secondary Ion Tandem Mass Spectrometry," *New Horizons in*

Neonatal Screening, 1994. To increase the number and rate at which samples can be analyzed, the development of automated sample preparation, instrumental analysis, and data interpretation was required. The increase in sample throughput and the ease of sample preparation allows for the more efficient and exacting diagnosis of a great number of metabolic disorders, a process necessary in determining the health of a newborn baby, or, for that matter, anyone in clinical care. The ranges of clinical symptoms and abnormalities in simple blood tests are so extreme that extensive biochemical investigation is warranted whenever metabolic disease is suspected, as noted in Millington, et al., "Diagnosis of Metabolic Disease," from *Biological Mass Spectrometry: Present and Future,* 3.15, 1994.

Metabolic profiling of amino acids and acylcarnitines from blood spots by use of automated electrospray tandem mass spectrometry (ESI-MS/MS), is a more powerful diagnostic tool for inborn errors of metabolism. See Rashed, et al., *Clinical Chem.* 43:7, 1129-1141. New approaches to sample preparation and data interpretation have helped establish the methodology as a robust, high-throughput neonatal screening method. Compared with older methods, ESI-MS/MS is much more versatile and less labor intensive, because most of the steps can be automated.

Inborn errors of metabolism usually result from defective enzymes or cofactors. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is a very common disorder of fatty acid oxidation. As seen in Chace et al., *Clin. Chem.*, 43:11, 2106-2113, MCAD deficiency is diagnosed on the basis of the increase of medium chain length acylcarnitines, as identifiable by isotope dilution mass spectrometry methods. Butyl esters of acylcarnitines share a similar fragmentation pattern with a common fragment ion at 85Da after collision-induced dissociation using a mass spectrometer. The fragmentation pattern differences are compared to known

spectra of healthy individuals and thereby can be diagnosed. In a clinical setting, analysis of acylcarnitines by tandem mass spectrometry is possible as their associated methyl esters allow the diagnostic recognition of all patients with MCAD deficiency, regardless of the underlying mutation, symptomatic state, or treatment. Also, the analysis of amino acids as their associated butyl esters has been validated for newborn screening of phenylketonuria (PKU), tyrosinemia, maple syrup urine disease, and homocystinuria, all of which, among others, are detected by mass spectrometry.

The most selective and sensitive spectrometry, as it relates to genetic disorders, is performed by the automated, electrospray tandem mass spectrometer. The use of ESI-MS/MS has been presented to successfully and quickly provide a specific and accurate screening method (Rashed, et al.). The method itself, however, must be complemented with an efficient sampling procedure and optimized injection and scan function mode to accommodate, with utmost accuracy, many samples at one time, thereby maximizing throughput while maintaining sensitivity and accuracy.

The efficiency of the ionization of the compounds is very high with the implementation of electrospray ionization. As seen in U.S. Patent No. 5,352,891, Monning et al., the high ionization efficiency allows useful spectra required for even very small quantities of material. In other words, electrospray tandem mass spectrometry is very sensitive and specific in regards to its compound injection systems, thereby allowing a more broad spectrum of diseases to be covered, a lower false positive rate to be achieved, high specificity to be obtained, and shortened analytical time permitted. The use of the electrospray tandem MS/MS has been shown to increase throughput. Moreover, the technique has been successfully applied to prenatal diagnosis (Rashed, et al., 1130) and other screening processes. However, optimization of the

method of screening newborns must be achieved by maximizing sample throughput in the most efficient and accurate way, beginning in the sample preparation, and culminating with the quality assurance. The overall process lends itself to parental peace-of-mind, and expedient and cost-effective results.

Sample preparation in support of the genetic screening of an individual for carnitines and α-amino acids (genetic markers for inborn errors in metabolism) for use in mass spectrometry is seen in the art. The standard method of collecting samples for neonatal screening is a heel prick followed by depositing the whole blood on special filter paper (or Guthrie cards) as a series of spots. See Millington, et al., *International Journal of Mass Spectrometry and Ion Processes*, 111, 212, 1991. The latest developed method of preparing the butyl ester derivatives of acylcarnitines and amino acids from the blood spots consists of processing samples in microplates. An automated blood-spot puncher punches a single blood spot from each Guthrie card directly into the individual wells of the microplate. To the blood spot punch in each well a methanolic solution containing known concentrations of stable isotope-labeled standards is added. The label standards might include glycine and alanine; valine, methionine, and phenylalanine; leucine and tyrosine; ornithine; carnitine; acetylcarnitine; propionylcarnitine; octanoylcarnitine; and palmitoylcarnitine, all in combination in some concentration as to enhance the sensitivity for particular compounds, as required by respective testing protocol. The samples are extracted and the extracts are then transferred to another microplate where the methanol is removed through evaporation. To the residue in each well, butanolic HCl or other chemical modifiers are added and the derivatization is completed by heating. Final residues are reconstituted and placed in an autosampler tray for introduction into the MS.

The incorporation of isotope-dilution techniques as standards provides quantitative information for specific components of each sample. There is the need for an optimal concentration of a combination of 12 amino acid standards and 8 acylcarnitine/carnitine standards to improve accuracy and provide for quality control, as well as to provide for a number of scan functions that maximize metabolite information with high-throughput. Quality control and quality assurance in a clinical environment is of utmost importance because of the method and instrumentation that has evolved for the optimization of sample throughput. It is especially important as mass spectrometry results are correlated to the general populations of newborns so as to show accurate results in demographic trends.

The advantages of ESI-MS/MS over alternative methods of analysis are its high specificity and accuracy of quantification through use of the isotope-dilution technique, plus its speed and amenability to automation. See Chace et al., *Clin. Chem.* Vol. 39, No. 1, 1993. Coupling the sensitivity in detection with the requirement that newborn screening requires rapid throughput, high accuracy, high precision, high selectivity, and a high value to low cost ratio, there is now a need in the clinical environment, now satisfied by the present invention, for an accurate means of assuring the quality of data for genetic disorder diagnosis is obtained in an organized and accurate manner. This quality can be coupled to the most efficient method of preparing and scanning samples, so as the number of false-positives and false-negatives are reduced, and sample throughput is necessarily maximized in the diagnostic clinical setting.

U.S. Patent No. 5,538,897, July 23, 1996 (Yates, III et al.) shows a method for correlating a peptide fragment mass spectrum with amino acid sequences derived from a database. A peptide is analyzed by a tandem mass spectrometer to yield a peptide fragment mass spectrum. A protein sequence database or a nucleotide sequence database is used to predict one

or more fragment spectra for comparison with the experimentally derived fragment spectrum. The various predicted mass spectra are compared to the experimentally derived fragment spectrum using a closeness-of-fit measure, preferably calculated with a two-step process, including a calculation of a preliminary score and, for the highest-scoring predicted spectra, calculation of a correlation function.

U.S. Patent No. 5,206,508, April 27, 1993 (Alderdice et al.) teaches a tandem mass spectrometry system, capable of obtaining tandem mass spectra for each parent ion without separation of parent ions of differing mass from each other. This system would in addition provide the capability to select a particular ion prior to excitation.

U.S. Patent No. 5,352,891, October 4, 1994 (Monning et al.) demonstrates the production of mass spectra of chemical compounds of high molecular weights having a multiplicity of peaks is improved by generating an enhanced mass spectrum from the observed mass-to-charge spectrum. Signal-to-noise ratio can in some applications be improved by including in the product all portions within the discrete peaks in the mass-to-charge spectrum, which are contained within a window around each of the discrete peaks.

DISCLOSURE OF INVENTION:

It is the objective of the present invention to improve the method of screening newborns by implementing efficient sampling protocols and data quality controls. As initial and final steps to the use of electrospray tandem mass spectrometry for inborn metabolite error screening, the sample efficiency and quality assurance will complement a more rapid sample throughput method with a high value to low cost ratio. All values are compared to known thresholds as a means for evaluating the contents of the sample. High accuracy and high precision found in a large number of samples will quickly provide consistent diagnosis at the clinical level.

Electrospray tandem mass spectrometry is very sensitive and specific and can detect a broad spectrum of disorders at the genetic level. The already shortened analytical time and high specificity increases the rate at which samples that can be analyzed. Including internal standards in the sample preparation that decrease extraction error and allow for mixed mode scan functions further increases sample throughput. The internal standards are used to provide the quantitative information needed to detect specific components. Use of proper ratios of each particular ion enables the detection of many metabolites at one time, thereby eliminating duplicate analysis, allowing secondary runs to be used for quality assurance and proficiency testing rather than for detection of preliminary compounds.

It is a secondary objective of the present method to include EDTA standards that can determine whether or not the blood was collected properly. Contaminated blood or blood collected from tubes rather than a heel prick spot is improper and identifiable by this standard.

It is a third objective of the present method to include quality assurance standards such as $^{2}H_3$ - Serine (deuterium 3 labeled Serine) to show the computer is recognizing normally unfounded compounds. Serine is an amino acid that is not included or recognized in a normal scan, so $^{2}H_3$ - Serine is added to an acylcarnitine scan to show that, when this compound is detected and shown as a peak, the computer is capable of detecting foreign compounds. In effect, drug-ridden or contaminated samples may be flagged.

It is a fourth objective of the present method to include proper correction factors, mass values, quality assurance flags, and sample preparation flags as input values, complementing a database that is used for checking calculations as produced using a spreadsheet, thereby insuring accurate data reduction. This provides enhanced quality assurance. When an abnormal sample is

noted, a recommended action is to be taken. Database storage of values facilitates disease rate data reporting, trend generation and analysis, total sample-per-day values, and QA/QC analyses.

It is a fifth objective of the present method to include a quality control step that uses unlabeled standards and control blood standards to assure the consistency and accuracy in the detection of the twenty metabolites.

BRIEF DESCRIPTION OF THE DRAWINGS:

FIG. 1 is a simplified block diagram showing the overall methodology. Five principle processes are correlated from sample preparation to system diagnostics.

FIG. 2 is a block diagram showing in more detail the steps involved in preparing the sample.

FIG. 3 is a block diagram showing in more detail the steps involved in the automated use of an electrospray tandem mass spectrometer to include the use of proper scan functions to maximize accurate output.

FIG. 3a is a spreadsheet showing the possible upper or lower thresholds used to determine which samples are to be flagged for further decision-making or re-testing.

FIG. 3b is an example of a Free Carnitine MRM scan, showing the pertinent peaks and values for quality assurance.

FIG. 3c is an example of an Acetylcarnitine MRM scan, showing the pertinent peaks and values for quality assurance.

FIG. 3d is an example of a full scan Acetylcarnitine profile, showing the pertinent peaks and values for quality assurance.

FIG. 3e is an example of a full scan Amino Acid profile, showing the pertinent peaks and values for quality assurance.

FIG. 3f is an example of a basic Amino Acid MRM scan, showing the pertinent peaks and values for quality assurance.

FIG. 4 is a block diagram showing in more detail the steps involved in processing the data after acquisition of the values, which have been produced from the spectrometer.

FIG. 5 is a block diagram showing in more detail the steps involved in interpreting the data as it relates to demography and decision making.

FIG. 6 is a block diagram showing in more detail the steps involved in monitoring system diagnostics and implementing quality controls.

BEST MODE FOR CARRYING OUT THE INVENTION:

The invention will now be described in detail in relation to a preferred embodiment and implementation thereof which is exemplary in nature and descriptively specific as disclosed. As is customary, it will be understood that no limitation of the scope of the invention is thereby intended. The invention encompasses such alterations and further modifications in the illustrated device, and such further applications of the principles of the invention illustrated herein, as would normally occur to persons skilled in the art to which the invention relates.

FIG. 1 represents an overview of the method of screening newborns in the clinical diagnostic setting involving five main steps, each of which are important for rapid, automated, and accurate sample analysis. Efficient sample preparation **10** is necessary to insure accurate derivatization of the metabolites, and certain additives or internal standards are implemented and important to provide quantitative information for specific components of each sample. After sample preparation **10**, the samples are loaded into the electrospray tandem mass spectrometer **12**, which implements many automated features to insure the speed and consistency of sample scanning. Data is then acquired and processed to a reduced and organized form as seen in box

14. Values produced from the scan of the mass spectrometer are processed and printed into spreadsheet form to further allow checking of the calculations, a means of assuring accurate number production and quality. Acquired data is then interpreted by an assisted diagnostic interpretation system **16** which integrates the results with the demographic data related to the baby and allows for correlation to a specific disorder based on any noted peaks. The process, working in conjunction with software, allows for data reporting which is a way of monitoring daily output and assisting in necessary decision making for further action, such as follow-up, or re-testing. All spectra data is kept accurate using system diagnostic checks and quality control samples as seen in step **18**. To assure diagnostic accuracy and sample quality, periodic system integrity checks and control samples that include specific additives are employed. In combination, the above mentioned steps maximize the rate and quality at which newborn blood samples are screened for metabolic disorders, which is necessary in the clinical setting.

Fig. 2 shows an overview of the sample preparation procedure (step 1 of FIG. 1). An initial sample login **20** is performed by coding each sample, thereby associating the sample to a specific location in a microtiter well. The samples consist of blood spots placed on designated areas of filter paper. The spots are punched with a diameter in the range of 3/16 in. to 1/8 in. and placed into the designated microtiter well. Internal standard preparations **22** are prepared in methanol to produce an extraction solvent, which is added to the dry blood spot in each well. Extraction solvent additions **24** are performed using automated sample handling equipment.

The methanol serves as the solvent extraction medium while the internal standards serve to quantify the metabolites in the dry blood matrix. The internal standard preparations **22** comprise an ideal mix of twenty stable isotopes - twelve amino acid standards and eight acylcarnitine/carnitine standards. A list of the amino acid standards can be found in FIG. 2a.

The left column shows the standard concentrations of the concentrated working stock **20a**. The stock solution is diluted 1:100 v/v with methanol to produce concentrations of daily working standards **22a**. The concentrations of the daily working standards **22a** can be adjusted to analyze two 3/16", two 1/8" or a single 1/8" dried blood spots by adjusting the volume of the extraction solvent additions **24** (FIG. 2) or the concentration of the working stock **20a**. The daily working standards **22a** serve as both the extraction solvent and the means for internal standardization of the analysis.

Free Carnitine and Acylcarnitine internal standards are listed in FIG. 2b. Again, the left column lists the concentrations of the working stock **20b** used in the dilution with methanol 1:100 v/v, to produce the daily working standards **22b.** Also, the daily working standards **22b** can be adjusted as described above for the blood spot analysis.

Both groups of standards are provided in the extraction medium for the optimum mixed mode scan functions, which maximize metabolite detection. The metabolite groups detected include the α-amino acids – alanine, phenylalanine, tyrosine, glutamic acid, ornithine, citrulline, arginine – and the carnitines – free carnitine, acylcarnitines, acetylcarnitine, octanoylcarnitine, palmitoylcarnitine.

Now following FIG. 2, after extraction solvent addition **24**, the solvent is transferred at step **26** to a plate, or microtiter plate, having rounded-bottom wells where the solvent is removed using a nitrogen drying system at step **27**. The blood extract then undergoes esterification and is chemically modified and heated at step **28** to become a derivative. Excess derivative is removed at step **29** and a mobile phase solvent is added using an automated sample handling system. Plate seals retard any solvent evaporation.

FIG. 3 shows the steps involved after the sample is prepared and standards are included and made ready for introduction into the automated electrospray tandem mass spectrometer. Optimization of the MS/MS systems **30** is achieved by using a tuning solution, and the electrospray MS/MS system **32** is a low flow rate system employing the use of a fused silica line displaced to the tip of the electrode. Automated injection systems **34** use the fused silica line to directly connect the injector to electrode tip to minimize dead space. The scans implemented to detect the necessary fragments of the ions consist of five mixed-mode scan functions **36** for maximizing metabolite and quality assurance information. The mixed-mode scan functions **36** include free carnitine MRM, acetylcarnitine MRM, full scan acylcarnitine, full scan amino acids, and basic amino acid MRM, whereas a full scan covers a wider range of mass to charge ratios, thereby a wider range of peaks can be compared. Each peak corresponds to a concentration or threshold number and compared to a known upper or lower threshold.

Examples of the values of the thresholds can be seen in FIG. 3a. It should be understood that all sample values necessary in metabolic error determination or quality assurance falling above or below a certain threshold are flagged, or identified, for diagnostic purposes, re-testing, or other clinical decision making.

FIG. 3b demonstrates a Free Carnitine MRM implementing quality assurance. An MRM is a scan for a particular compound showing dual masses **401** (parent mass and daughter mass respectively). A first peak **403** is detected as the free carnitine fragments. The resulting concentration of Free Carnitine **405** is then given. Quality is assured in this scan by looking at the d_3 free CN (deuterium 3 free carnitine) peak **404** which comes from the hydrolysis of d_3 labeled acylcarnitines. The resulting "hydrofree" concentration value **409** is a quality assurance

flag for acylcarnitine hydrolysis and is also a correction for true concentrations of Free Carnitine **405.**

FIG. 3c demonstrates an Acetylcarnitine MRM. Peak **501** is the acetylcarnitine (acetylCN) peak and peak **503** is a quality assurance (QA) peak manifesting the hydrolysis of glutamate. The resulting glutamate concentration **505** shows the amount of interference from a glutamate, which is corrected for in the acetylCN concentration **504** determination. Other QA checks for propionyl CN are implemented in this scan as duplicate peaks **507** and **509**.

A profile of the Acylcarnitine full scan is shown in FIG. 3d. Added internal standards are fragmented and revealed as peaks **601, 602, 603, 604, 605.** A list of the concentrations of the detectable metabolites **610** is then provided as well as the molar ratios **612**. A QA test is included in this scan as a bad derivative value **614** which stems from any peak found around a m/z, amu value of 403. The bad derivative value **614** would reveal poor sample preparation if elevated. An EDTA QA flag **616** is also implemented to reveal sample collection method. Elevated values of the EDTA QA flag **616** manifest samples drawn from tubes rather than heel pricks, or reveal lengthy preservation maintenance.

Another QA method is used in this scan, revealed by an intensity value **618**. An elevated intensity value shows the sample was scanned with adequate sensitivity. If the intensity value **618** is too low, the sample will be flagged (noted), and the sample may be re-tested depending on the protocol.

FIG. 3e is an example of a full scan Amino Acid analysis. Amino acids in the internal standards fragment and are shown as peaks **710, 711, 712, 713, 714, 715, 716, 717**. Amino Acid concentration values **701** are listed, along with a QA flag value **703** at around a m/z, amu value of 165. The QA flag value **703** would most likely be produced from the addition of $^{2}H_{3}$ - Serine ,

which would be added in a sample to manifest proper detection of compounds normally not found in a routine sample, as Serine is an amino acid not included in the list of amino acids relevant to any disorders. An intensity flag **705** is also implemented to show adequate sensitivity in detection.

FIG. 3f is an example of a basic Amino Acid MRM. The QA flag occurs at peak **802**, and the scan includes duplicate Citrulline analysis **804,** normally peaking around a m/z value of 215 and 232.

FIG. 4 describes the processing of the data acquired from the scan functions used for the mass spectrometer. Step **40** is the input of all mass values, constants for concentration calculations, correction factors for extraction efficiency, ratios of concentration data, and cut-off values. Quality assurance flags, sample preparation flags, and sensitivity flags are also inputted. The flags include the above described peaks, intensity values, bad derivative values, and EDTA values, and are important because they reveal whether or not the samples are contaminated or drug-ridden, and they are very telling of how the samples were contained, or from where the samples were drawn. Also, they assist in maintaining instrument accuracy and consistency. The results are processed and printed for step **42**. The scan functions described for FIGs. 3a-3e can detect multiple diseases based on the fragments of the metabolites detected. The revealing peaks will eventually lead to the profiles noted in boxes **43a** and **43b**. The profiles may include the noting of peaks picked up using the quality assurance or quality control standards as well.

FIG. 5 shows the steps involved in interpreting the organized data. The spreadsheet data is inputted to a database module for recognition of the file and sample types. As seen in step **50**, the data is interpreted so parameters can be assigned to the particular sample, and the results given. The results are then integrated in step **52** with demographic data of the newborn. The

demographics may include age, type of specimen, or other notation such as whether or not the baby is premature, etc. Samples that show an abnormality, or seem to show a revealing peak, are flagged to be interpreted using a reference guide and decisions are made on the next course of action as step **54**. Referencing the decision tree and recommending action would be the next step as step **56**. The flagged samples are correlated with the database module used to distinguish abnormal peaks, and a decision to re-test or diagnosis is made. In step **58**, as a measure of quality assurance and quality control, the days mean sample and trend generation is recorded to follow the statistical occurrences of diseases, and to maintain high-throughput sampling. This includes automated data reporting and internet communication reporting.

FIG. 6 shows the steps involved in further maintaining quality assurance using quality control samples and maintaining system integrity. Quality control samples are prepared as step **60**. The samples consist of QA blood spots and liquids prepared as unlabeled standards at the same concentrations as the internal standards, and scanned. The control blood standards implemented in this step **60** consist of hemolyzed blood, EDTA, and $^{2}H_{3}$ - Serine, or some other recognized marker. These are run and compared to standards that consist of hemolyzed blood, EDTA, $^{2}H_{3}$ - Serine, and one of the twenty compounds that are the same as those used in the internal standards, but unlabeled. The computer is properly set up to recognize and interpret the results. Another step in maintaining quality assurance is provided as step **61**. Systems are monitored in a database program to detect changes in system integrity or sensitivity. A final step in maintaining system diagnostics is included as step **63**. Maintenance methods and schedules are constantly followed and monitored through archival systems and via the Internet through ongoing monitoring of mass spectrometry data.

INDUSTRIAL APPLICABILITY:

The invention is used to assist in the diagnosis of the many metabolic disorders that must be detected and treated within days after a birth to assure a baby develops normally and/or survives. At the clinical level, testing must be performed at a high throughput rate, while being accurate and precise. Any result, whether positive or negative, must also be coupled to stringent quality controls to assure such accuracy and precision. It is envisioned that the invention will be the fundamental screening process for assisting in the diagnosis of all neonatal disorders occurring at the genetic level.

CLAIMS IN THE INVENTION:

I claim:

1. A method of screening newborns utilizing a system that employs an electrospray tandem mass spectrometer, comprising the steps of:

receiving a plurality of blood spots;

combining an amino acid standard and a free carnitine/acylcarnitine standard to form an internal standard containing a plurality of labeled compounds;

preparing a plurality of samples, each of said samples consisting essentially of said internal standard, methanol, and a blood extract from one of said blood spots;

introducing said plurality of samples into said electrospray tandem mass spectrometer by means of an automated injection system, wherein each of said samples are scanned using multiple mixed-mode scan functions, wherein each of said scan functions include a means of quality assurance, and thereby producing a plurality of scan results, each of said scan results having said scan data;

analyzing each of said scan results;

preparing a plurality of control blood samples, each of said control blood samples consisting essentially of hemolyzed blood, EDTA, and $^{2}H_{3}$ – Serine;

preparing a plurality of standards, each of said standards consisting essentially of hemolyzed blood, EDTA, $^{2}H_{3}$ – Serine, and one of said labeled compounds;

scanning said plurality of control blood samples to produce control sample results;

scanning said plurality of standards to produce a plurality of standard results; and,

comparing said control sample results obtained for each of said quality control samples to said plurality of standard results obtained for each of said standards.

2. The method of claim 1, wherein each of said samples is prepared in a rounded bottom well of a microtiter plate.

3. The method of claim 2, further comprising the step of coding each of said samples to associate each of said samples to said rounded bottom well.

4. The method of claim 1, wherein said mixed-mode scan functions include a free carnitine MRM, an acetylcarnitine MRM, an acylcarnitine full scan, an amino acid full scan, and a basic amino acid MRM.

5. The method of claim 1, further comprising the steps of:

organizing said scan data by means of a spreadsheet;

inputting said scan data acquired from each of said scan results into a database adapted to assist in organizing, recognizing, and interpreting said scan data;

assigning a plurality of scan results with demographic data of each of said newborns corresponding to each of said samples;

flagging each of said samples that reveal an abnormality, thereby forming a plurality of flagged samples, wherein each of said flagged samples can be interpreted using a reference guide, and wherein a next course of action can be taken for each of said flagged samples.

6. The method of claim 1, further comprising the step of monitoring said system to detect changes in system integrity or sensitivity.

7. A method of screening newborns utilizing a system that employs an electrospray tandem mass spectrometer, comprising the steps of:

receiving a plurality of blood spots;

combining an amino acid standard and a free carnitine/acylcarnitine standard to form an internal standard containing a plurality of labeled compounds;

preparing a plurality of samples, each of said samples consisting essentially of said internal standard, methanol, and a blood extract from one of said blood spots;

scanning said plurality of samples using said electrospray tandem mass spectrometer to produce scan results;

analyzing said scan results;

preparing a plurality of control blood samples, each of said control blood samples consisting essentially of hemolyzed blood, EDTA, and $^{2}H_{3}$ – Serine;

preparing a plurality of standards, each of said standards consisting essentially of hemolyzed blood, EDTA, $^{2}H_{3}$ – Serine, and one of said labeled compounds;

scanning said plurality of control blood samples to produce control sample results;

scanning said plurality of standards to produce a plurality of standard results; and,

comparing said control sample results obtained for each of said quality control samples to said plurality of standard results obtained for each of said standards.

8. The method of claim 7, wherein said amino acid standard contains twelve labeled amino acids.

9. The method of claim 8, wherein said twelve labeled amino acids are selected from the group consisting of $^{15}N^{13}C$ – Glycine, $^{2}H_4$ – Alanine, $^{2}H_8$ – Valine, $^{2}H_3$ – Leucine, $^{2}H_3$ - Methionine, $^{2}H_5$ – Phenylalanine, $^{2}H_4$ – Tyrosine, $^{2}H_3$ – Aspartate, $^{2}H_3$ – Glutamate, $^{2}H_2$ – Ornithine-2HCl, $^{2}H_2$ – Citrulline, and $^{2}H_4{}^{13}C$ – Arginine-HCl.

10. The method of claim 7, wherein said free carnitine/acylcarnitine standards contain eight labeled carnitines.

11. The method of claim 10, wherein said eight labeled carnitines are selected from the group consisting of $^{2}H_9$ – carnitine, $^{2}H_3$ – acetylcarnitine, $^{2}H_3$ – propionylcarnitine, $^{2}H_3$ – butyrylcarnitine, $^{2}H_9$ – isovalerylcarnitine, $^{2}H_3$ – octanoylcarnitine, $^{2}H_9$ – myristoylcarnitine, $^{2}H_3$ – palmitoylcarnitine.

12. The method of claim 7, wherein for the step of scanning said samples, a free carnitine MRM scan function is implemented.

13. The method of claim 12, wherein said free carnitine MRM scan function implements quality assurance data for acylcarnitine hydrolysis.

14. The method of claim 13, wherein said quality assurance data for acylcarnitine hydrolysis is a dual mass peak seen around 221.3/103.1 atomic mass units.

15. The method of claim 7, wherein for the step of scanning said samples, an acetylcarnitine MRM scan function is implemented.

16. The method of claim 15, wherein said acetylcarnitine MRM scan function implements quality assurance data for glutamate hydrolysis and two quality assurance flags for propionylcarnitine.

17. The method of claim 16, wherein said quality assurance data for glutamate hydrolysis is a dual mass peak seen around 261.3/85.1 atomic mass units.

18. The method of claim 7, wherein for the step of scanning said samples, an acylcarnitine full scan is implemented.

19. The method of claim 18, wherein said acylcarnitine full scan implements quality assurance data for a bad derivative value, whereby a poor preparation of said samples is revealed, and wherein said acylcarnitine full scan implements quality assurance data for EDTA, whereby said sample can be identified as being scanned with an adequate sensitivity.

20. The method of claim 7, wherein for the step of scanning said samples, an amino acid full scan is implemented.

21. The method of claim 20, wherein said amino acid full scan implements quality assurance data for amino acid detection accuracy.

22. The method of claim 21, wherein said quality assurance data for amino acid detection accuracy is a concentration value corresponding to an amount of $^{2}H_3$ – Serine.

23. The method of claim 7, wherein for the step of scanning said samples, a basic amino acid MRM is implemented.

24. The method of claim 23, wherein said basic amino acid MRM includes quality assurance data for citrulline.

25. The method of claim 24, wherein said quality assurance data for citrulline is a peak seen around a dual mass value of 232.3/113.1 atomic mass units.

26. The method of claim 7, further comprising the step of determining a next course of action for diagnosing or re-testing said newborn.

27. A plurality of internal standard preparations used with an electrospray tandem mass spectrometer to genetically screen newborns, comprising a mix of amino acid standards and acylcarnitine standards.

28. The internal standard preparations of Claim 27, wherein said amino acid standards are labeled standards selected from the group consisting of $^{15}N^{13}C$ – Glycine, $^{2}H_4$ – Alanine, $^{2}H_8$ – Valine, $^{2}H_3$ – Leucine, $^{2}H_3$ - Methionine, $^{2}H_5$ – Phenylalanine, $^{2}H_4$ – Tyrosine, $^{2}H_3$ – Aspartate, $^{2}H_3$ – Glutamate, $^{2}H_2$ – Ornithine-2HCl, $^{2}H_2$ – Citrulline, and $^{2}H_4{}^{13}C$ – Arginine-HCl.

29. The internal standard preparations of Claim 27, wherein said acylcarnitine standards are labeled standards selected from the group consisting of $^{2}H_9$ – carnitine, $^{2}H_3$ – acetylcarnitine, $^{2}H_3$ – propionylcarnitine, $^{2}H_3$ – butyrylcarnitine, $^{2}H_9$ – isovalerylcarnitine, $^{2}H_3$ – octanoylcarnitine, $^{2}H_9$ – myristoylcarnitine, $^{2}H_3$ – palmitoylcarnitine.

30. The internal standard preparations of Claim 28, wherein working stock concentrations of each of said amino acid standards are 2500 umol/L for said $^{15}N^{13}C$ – Glycine, and 500 umol/L for each of said $^{2}H_4$ – Alanine, $^{2}H_8$ – Valine, $^{2}H_3$ – Leucine, $^{2}H_3$ - Methionine, $^{2}H_5$ – Phenylalanine, $^{2}H_4$ – Tyrosine, $^{2}H_3$ – Aspartate, $^{2}H_3$ – Glutamate, $^{2}H_2$ – Ornithine-2HCl, $^{2}H_2$ – Citrulline, and $^{2}H_4{}^{13}C$ – Arginine-HCl.

31. The internal standard preparations of Claim 29, wherein working stock concentrations of each of said acylcarnitine standards are 152.0 nmol/ml for said $^{2}H_9$ – carnitine, 38.0 nmol/L for said $^{2}H_3$ – acetylcarnitine, 7.6 nmol/ml for each of said $^{2}H_3$ – propionylcarnitine, $^{2}H_3$ –

butyrylcarnitine, $^2H_9$ – isovalerylcarnitine, $^2H_3$ – octanoylcarnitine, $^2H_9$ – myristoylcarnitine, and 15.2 nmol/ml for said $^2H_3$ – palmitoylcarnitine.

32. The internal standard preparations of Claim 30, wherein said working stock concentrations of each of said amino acid standards are diluted with methanol to form daily working concentrations of amino acid standards.

33. The internal standard preparations of Claim 31, wherein said working stock concentrations of each of said acylcarnitine standards are diluted with methanol to form daily working concentrations of acylcarnitine standards.

AMENDED CLAIMS

[received by the International Bureau on 07 November 2001 (07.11.01);
original claims 1-33 replaced by amended claims 1-23 (4 pages)]

1. A method of screening newborns utilizing a system that employs an electrospray tandem mass spectrometer, comprising the steps of:

receiving a plurality of blood spots;

combining an amino acid standard and a free carnitine/acylcarnitine standard to form an internal standard containing a plurality of labeled compounds;

preparing a plurality of samples, each of said samples comprising said internal standard, methanol, and a blood extract from one of said blood spots;

scanning said plurality of samples using said electrospray tandem mass spectrometer to produce scan results;

preparing a plurality of control blood samples, each of said control blood comprising hemolyzed blood, EDTA, and $^{2}H_{3}$ – Serine;

preparing a plurality of standards, each of said standards comprising hemolyzed blood, EDTA, $^{2}H_{3}$ – Serine, and one of said labeled compounds;

scanning said plurality of control blood samples to produce control sample results;

scanning said plurality of standards to produce a plurality of standard results; and,

comparing said control sample results obtained for each of said quality control samples to said plurality of standard results obtained for each of said standards; and,

analyzing said scan results utilizing said standard results and said control sample results.

2. The method of claim 1, wherein each of said samples is prepared in a rounded bottom well of a microtiter plate.

3. The method of claim 2, further comprising the step of coding each of said samples to associate each of said samples to said rounded bottom well.

4. The method of claim 1, further comprising the steps of:

organizing said scan results by means of a spreadsheet;

inputting said scan results into a database adapted to assist in organizing, recognizing, and interpreting said scan data;

assigning said scan results with demographic data of each of said newborns corresponding to each of said samples;

flagging each of said samples that reveal an abnormality, thereby forming a plurality of flagged samples, wherein each of said flagged samples can be interpreted using a reference guide, and wherein a next course of action can be taken for each of said flagged samples.

5. The method of claim 1, wherein said amino acid standard contains twelve labeled amino acids.

6. The method of claim 5, wherein said twelve labeled amino acids are selected from the group consisting of $^{15}N^{13}C$ – Glycine, $^{2}H_4$ – Alanine, $^{2}H_8$ – Valine, $^{2}H_3$ – Leucine, $^{2}H_3$ - Methionine, $^{2}H_5$ – Phenylalanine, $^{2}H_4$ – Tyrosine, $^{2}H_3$ – Aspartate, $^{2}H_3$ – Glutamate, $^{2}H_2$ – Ornithine-2HCl, $^{2}H_2$ – Citrulline, and $^{2}H_4{}^{13}C$ – Arginine-HCl.

7. The method of claim 1, wherein said free carnitine/acylcarnitine standards contain eight labeled carnitines.

8. The method of claim 7, wherein said eight labeled carnitines are selected from the group consisting of $^2H_9$ – carnitine, $^2H_3$ – acetylcarnitine, $^2H_3$ – propionylcarnitine, $^2H_7$ – butyrylcarnitine, $^2H_9$ – isovalerylcarnitine, $^2H_3$ – octanoylcarnitine, $^2H_9$ – myristoylcarnitine, $^2H_3$ – palmitoylcarnitine.

9. The method of claim 1, wherein for the step of scanning said samples, a free carnitine MRM scan function is implemented.

10. The method of claim 9, wherein said free carnitine MRM scan function implements quality assurance data for acylcarnitine hydrolysis.

11. The method of claim 10, wherein said quality assurance data for acylcarnitine hydrolysis is a dual mass peak seen around 221.3/103.1 atomic mass units.

12. The method of claim 1, wherein for the step of scanning said samples, an acetylcarnitine MRM scan function is implemented.

13. The method of claim 12, wherein said acetylcarnitine MRM scan function implements quality assurance data for glutamate hydrolysis and two quality assurance flags for propionylcarnitine.

14. The method of claim 13, wherein said quality assurance data for glutamate hydrolysis is a dual mass peak seen around 261.3/85.1 atomic mass units.

15. The method of claim 1, wherein for the step of scanning said samples, an acylcarnitine full scan is implemented.

16. The method of claim 15, wherein said acylcarnitine full scan implements quality assurance data for a bad derivative value, whereby a poor preparation of said samples is revealed, and wherein said acylcarnitine full scan implements quality assurance

data for EDTA, whereby said sample can be identified as being scanned with an adequate sensitivity.

17. The method of claim 1, wherein for the step of scanning said samples, an amino acid full scan is implemented.

18. The method of claim 17, wherein said amino acid full scan implements quality assurance data for amino acid detection accuracy.

19. The method of claim 18, wherein said quality assurance data for amino acid detection accuracy is a concentration value corresponding to an amount of $^{2}H_{3}$ – Serine.

20. The method of claim 1, wherein for the step of scanning said samples, a basic amino acid MRM is implemented.

21. The method of claim 20, wherein said basic amino acid MRM includes quality assurance data for citrulline.

22. The method of claim 21, wherein said quality assurance data for citrulline is a peak seen around a dual mass value of 232.3/113.1 atomic mass units.

23. The method of claim 1, further comprising the step of determining a next course of action for diagnosing or re-testing said newborn.

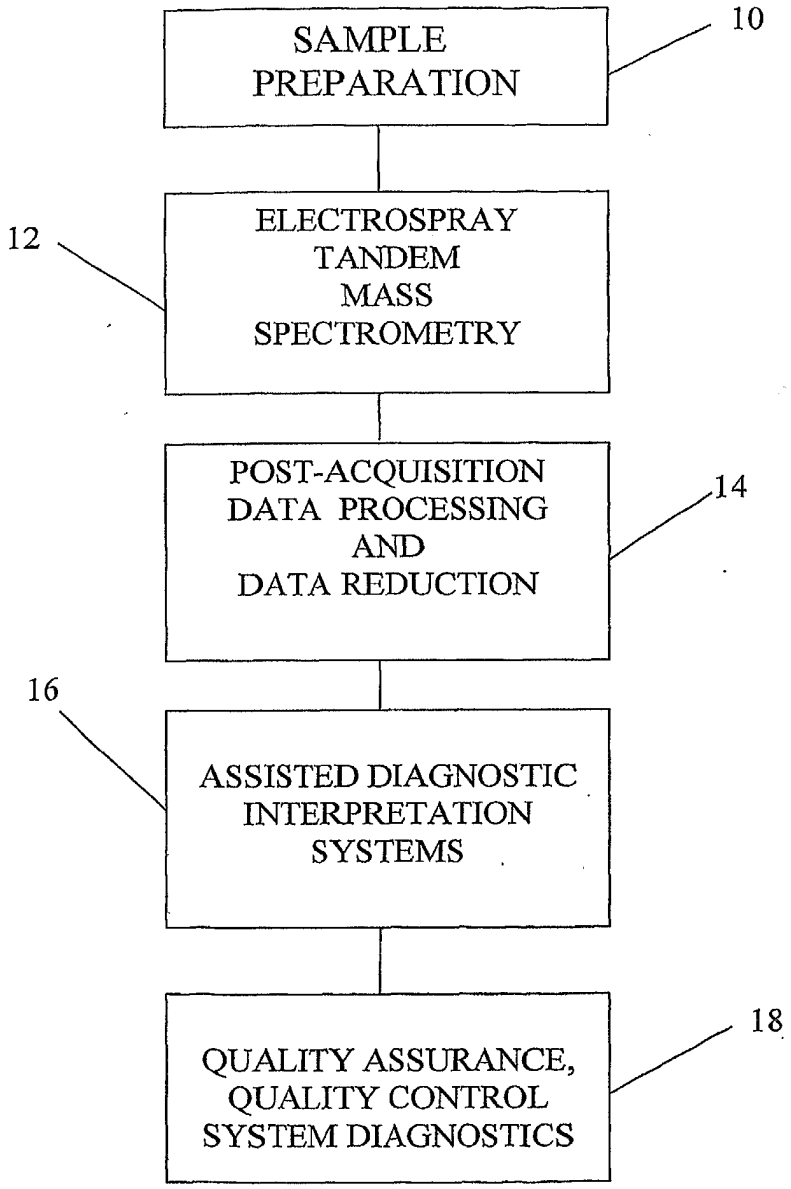

SAMPLE PREPARATION
10
ELECTROSPRAY TANDEM MASS SPECTROMETRY
12
POST-ACQUISITION DATA PROCESSING AND DATA REDUCTION
14
ASSISTED DIAGNOSTIC INTERPRETATION SYSTEMS
16
QUALITY ASSURANCE, QUALITY CONTROL SYSTEM DIAGNOSTICS
18

FIG. 1

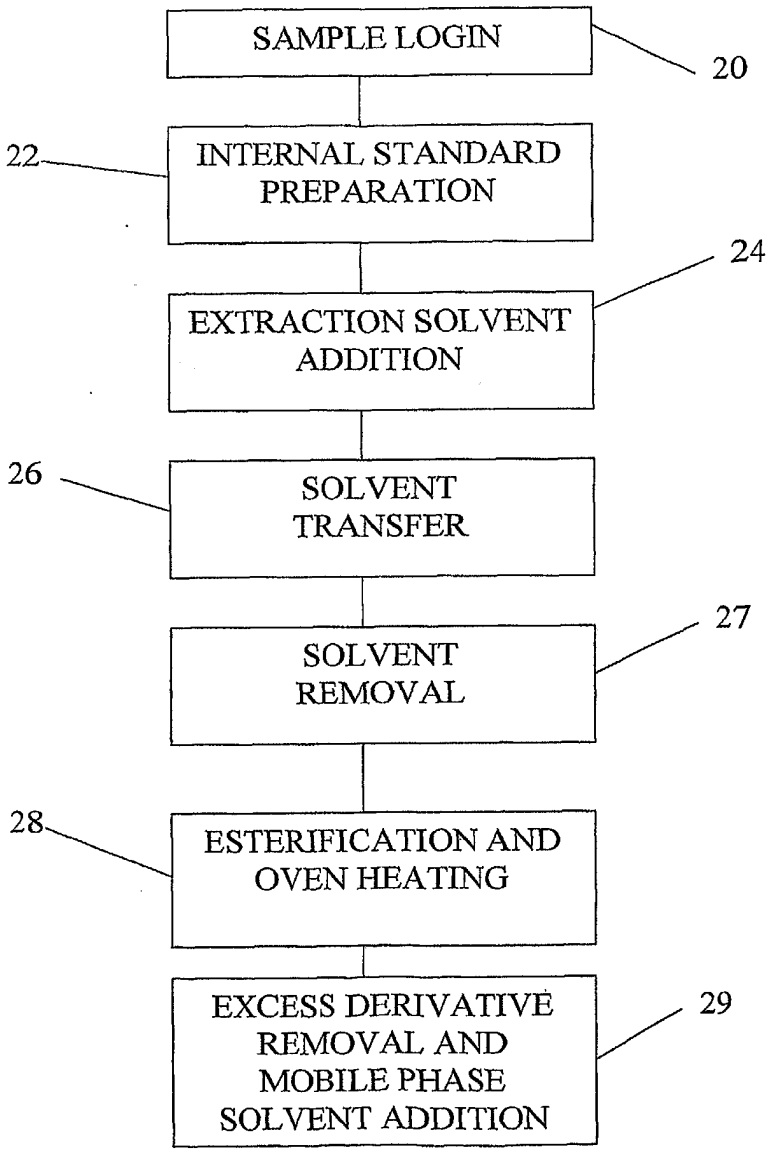

SAMPLE LOGIN
20
22
INTERNAL STANDARD PREPARATION
24
EXTRACTION SOLVENT ADDITION
26
SOLVENT TRANSFER
27
SOLVENT REMOVAL
28
ESTERIFICATION AND OVEN HEATING
EXCESS DERIVATIVE REMOVAL AND MOBILE PHASE SOLVENT ADDITION
29

FIG. 2

20a 22a

INTERNAL STANDARD	Concentration (umol/L)		Concentration (umol/L)
^{15}N, 2-^{13}C-Glycine	2500		12.5
$^{2}H_4$ - Alanine	500		2.5
$^{2}H_8$ - Valine	500		2.5
$^{2}H_3$ - Leucine	500		2.5
$^{2}H_3$ - Methionine	500		2.5
$^{2}H_5$ - Phenylalanine	500		2.5
$^{2}H_4$ - Tyrosine	500		2.5
$^{2}H_3$ - Aspartate	500		2.5
$^{2}H_3$ - Glutamate	500		2.5
$^{2}H_2$ - Ornithine 2HCl	500		2.5
$^{2}H_2$ - Citrulline	500		2.5
$^{2}H_4{}^{13}C$ - Arginine HCl	500		2.5

FIG. 2a

20b 22b

INTERNAL STANDARD	Concentration (nmol/ml)		Concentration (nmol/ml)
$^{2}H_9$-carnitine (free carnitine,CN)	152		0.76
$^{2}H_3$ - acetylcarnitine (C2)	38		0.19
$^{2}H_3$ - propionylcarnitine (C3)	7.6		0.04
$^{2}H_3$ - butyrylcarnitine (C4)	7.6		0.04
$^{2}H_9$ - isovalerylcarnitine (C5)	7.6		0.04
$^{2}H_3$ - octanoylcarnitine (C8)	7.6		0.04
$^{2}H_9$ - myristoylcarnitine (C14)	7.6		0.04
$^{2}H_3$ - palmitoylcarnitine (C16)	15.2		0.08

FIG. 2b

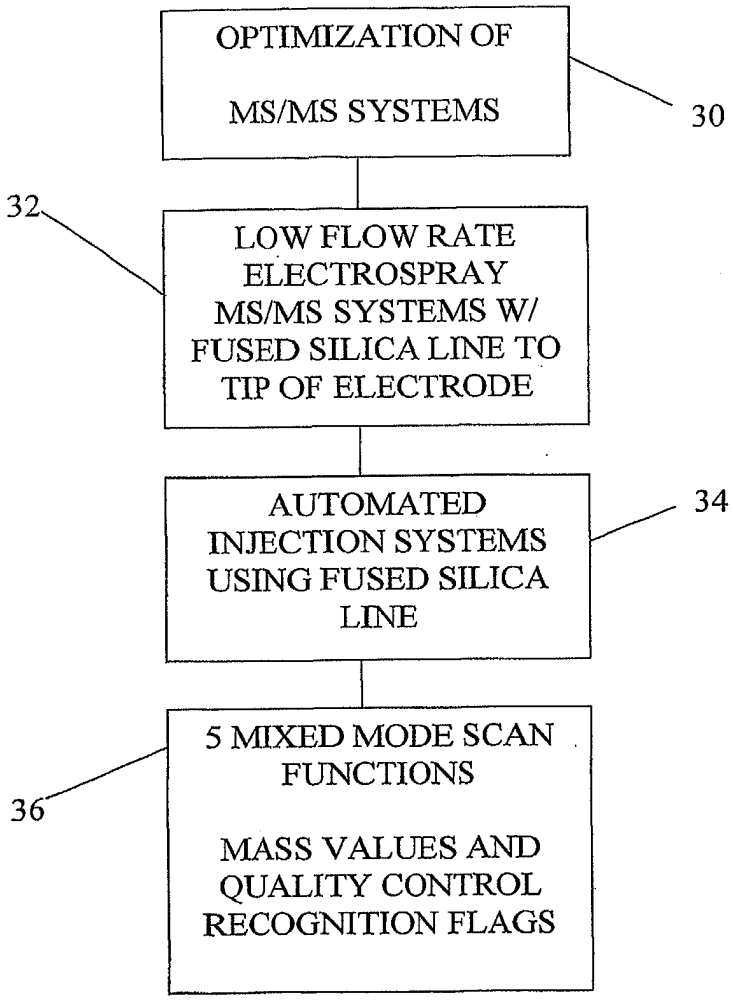

OPTIMIZATION OF
MS/MS SYSTEMS
30
32
LOW FLOW RATE ELECTROSPRAY MS/MS SYSTEMS W/ FUSED SILICA LINE TO TIP OF ELECTRODE
AUTOMATED INJECTION SYSTEMS USING FUSED SILICA LINE
34
5 MIXED MODE SCAN FUNCTIONS
MASS VALUES AND QUALITY CONTROL RECOGNITION FLAGS
36

FIG. 3

FIG. 3a

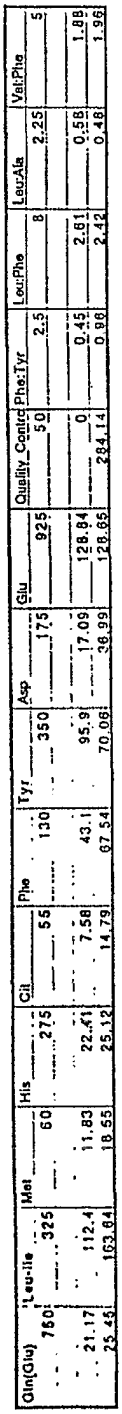
C4OH	C6	C5OH	BenzoylCN	C6OH	C8:1	C8	C8OH(C3DC)	C10:2	C10:1	C10	C4DC	C5DC(C10OH)	C12:1
1	0.32	0.9	1	0.5	0.8	0.35	0.32	0.32	0.32	0.42	0.8	0.14	0.75
0.11	0.05	0.08	0.01	0.04	0.04	0.03	0.02	0.05	0.04	0.02	0.14	0.02	0.06
0.12	0.02	0.14	0.03	0.02	0.1	0.05	0.02	0.07	0.08	0.09	0.3	0.03	0.06

Full Path	Filename	Sample Name	Failed Tests	FreeCN	Hydrofree	C2	C2Glutamate	C3mrm	C3/C2	C3	C4	C5:1	C5
		Upper Thresholds		350	7.5	80	25	5	0.3	4.5	1.75	0.5	1
		Lower Thresholds		10		4				0.2			
hard drive:Dat	ss9009450_cc	Sciex-1		22.1	1.49	8.54	2.75	1.3	0.15	0.88	0.19	0.04	0.13
hard drive:Dat	xqn99012712	Sciex-1	Quality Contro	47.05	1.56	6.28	2.19	1.39	0.22	0.91	0.1	0.06	0.15

C12	C5DC	C12OH	C14:2	C14:1	C14	C14OH	C16:1	C16	C16OH	C18:2	C18:1	C18	C12DC
0.9	0.25	0.25	0.4	1.1	1.3	0.25	1.5	10	0.22	3	8	4	2
0.1	0.02	5.36E-03	0.03	0.14	0.19	7.15E-03	0.18	2.82	0.02	0.11	1.31	0.98	0.16
0.04	0.02	0.02	0.06	0.12	0.11	0.02	0.12	0.79	0.02	0.33	1.45	0.81	0.12

C16:1OH	C18OH	Bad Derivative	EDTA	C8_C3	C3_C16	C8_C16	C5DC_C16	C14:1_C16	C4_C3	C8IS_INT	Gly	Ala	Val
0.22	0.24	0.3	0.2	1	2	0.28	0.12	0.28	1.5		750	1250	300
										400			
0.01	0.01	0.04	0.02	0.03	0.31	9.67E-03	8.79E-03	0.05	0.22	2240	143.97	192.28	81.19
0.02	0.02	0.04	0.15	0.06	1.16	0.06	0.03	0.15	0.11	1430	250.72	341.21	132.6

FIG. 3g



Gln(Glu)	Leu-Ile	Met	His	Cit	Phe	Tyr	Asp	Glu	Quality_Control	Phe:Tyr	Leu:Phe	Leu:Ala	Val:Phe
760	325		60	275	55	130	175	925	50	2.5	8	2.25	5
21.17	112.4	11.83	22.41	7.58	43.1	95.9	17.09	128.84	0	0.45	2.81	0.58	1.88
25.45	163.84	18.55	25.12	14.79	67.54	70.08	38.99	128.65	284.14	0.98	2.42	0.48	1.96

Met:Phe	Glu:Phe	PheIS_INT	Orn	Cit	HomoCit	Arg
1	8		175	55	60	100
0.05		2500				
0.27	2.99	37552	23.22	9.6	1.39	4.86
0.27	1.9	20168	23.28	16.29	0.97	2.51

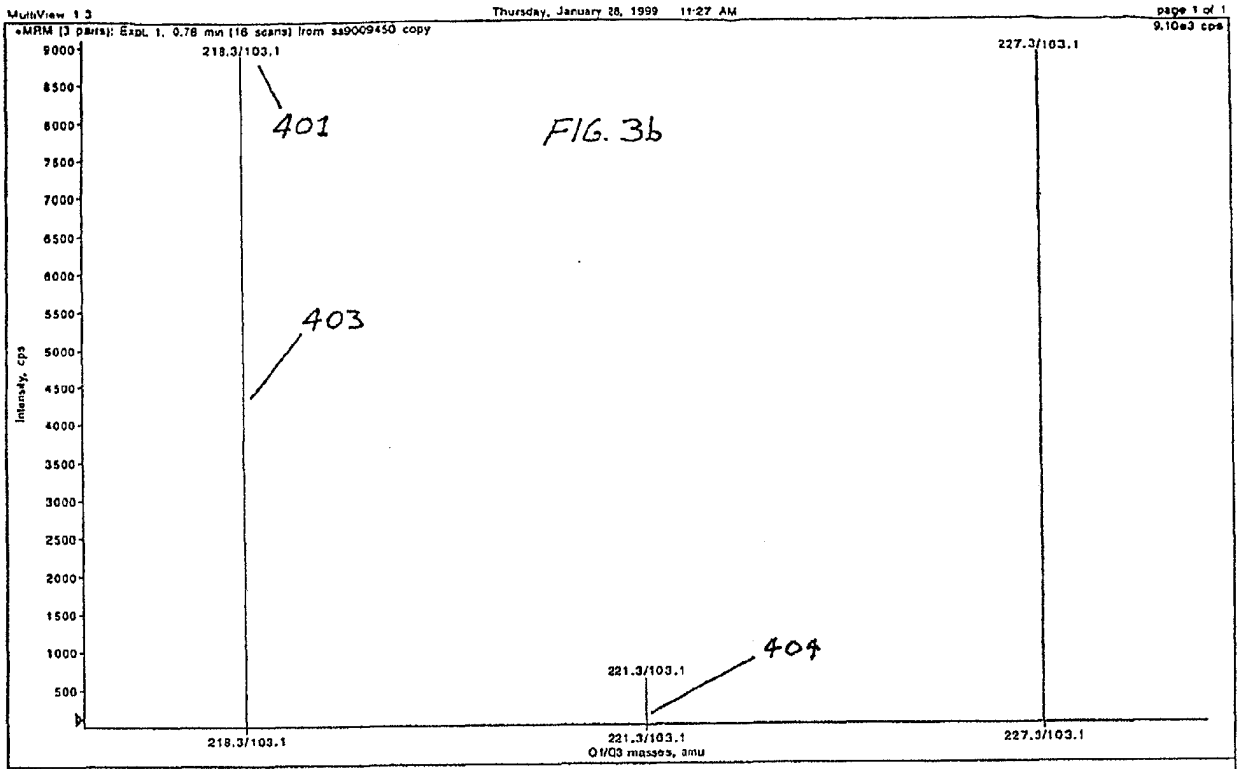


FIG. 3b

Scratch pane 40

hard drive:Desktop Folder:Patent:ss9009450 copy

Name	Result	Concentration
FreeCN	-	22.10
Hydrofree	-	1.49

405

Test	Result	Ratio

409

Mass	Result	Intensity

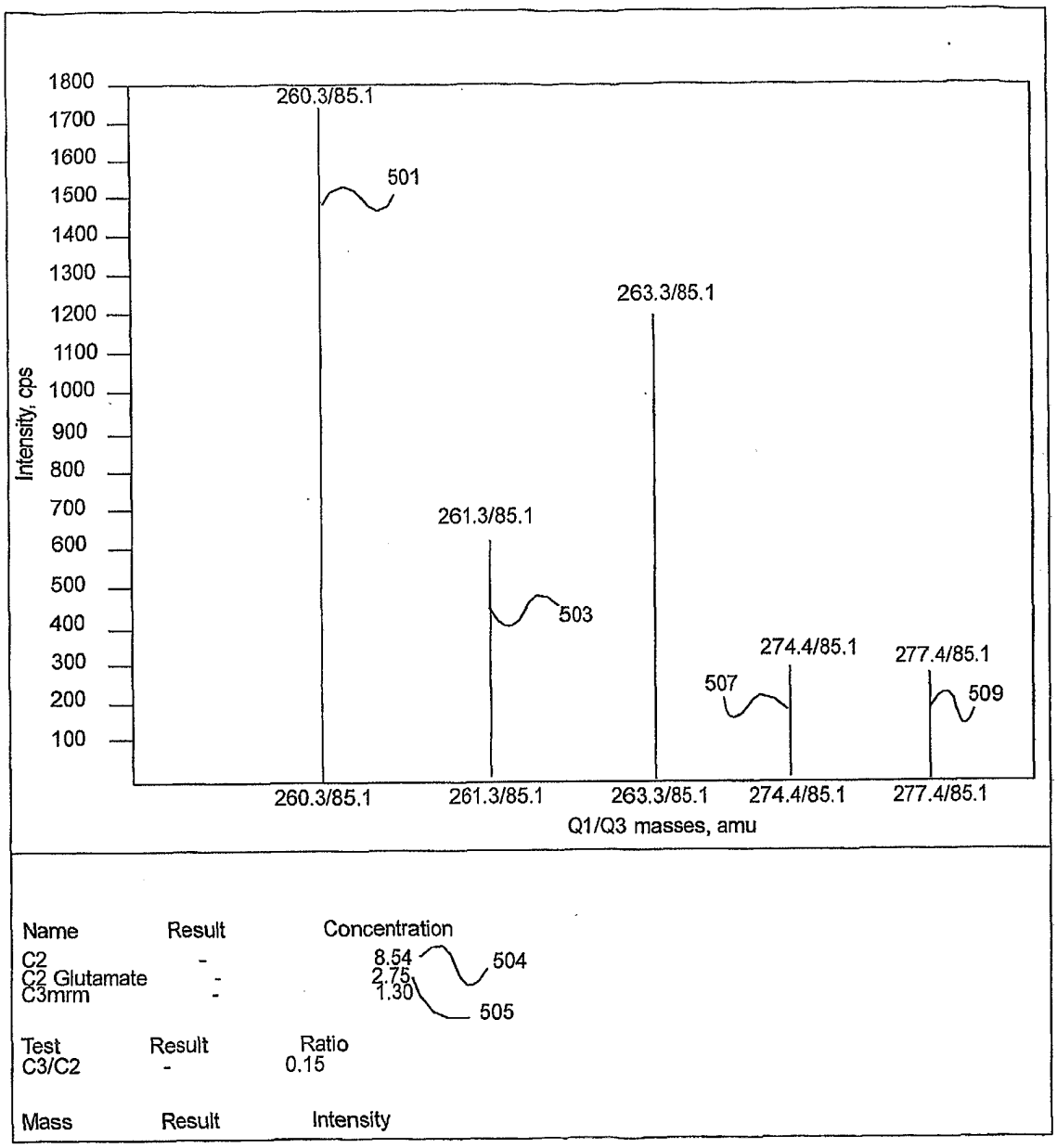
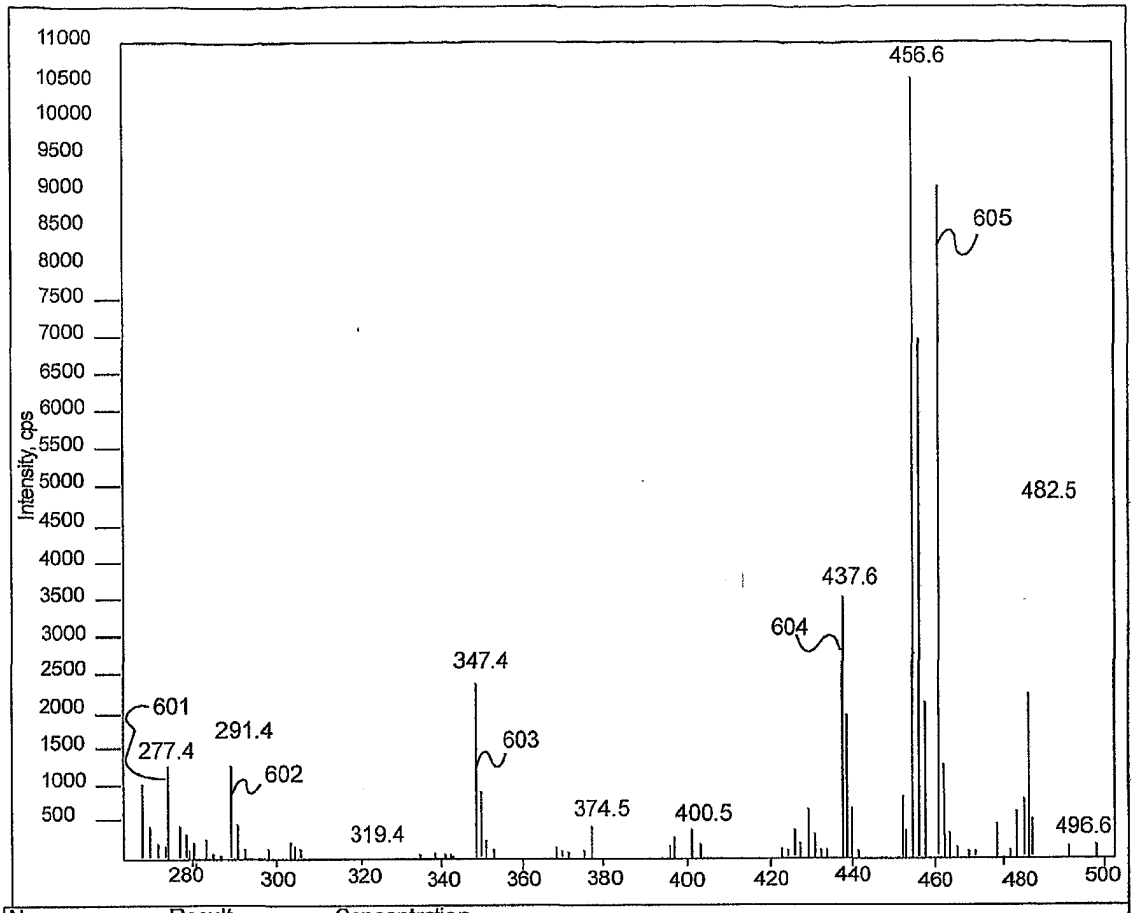

1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
Intensity, cps
260.3/85.1
501
263.3/85.1
261.3/85.1
503
274.4/85.1
507
277.4/85.1
509
260.3/85.1
261.3/85.1
263.3/85.1
274.4/85.1
277.4/85.1
Q1/Q3 masses, amu
Name Result Concentration
C2 -
C2 Glutamate -
C3mrm -
8.54
2.75
1.30
504
505
Test Result Ratio
C3/C2 - 0.15
Mass Result Intensity

FIG. 3c

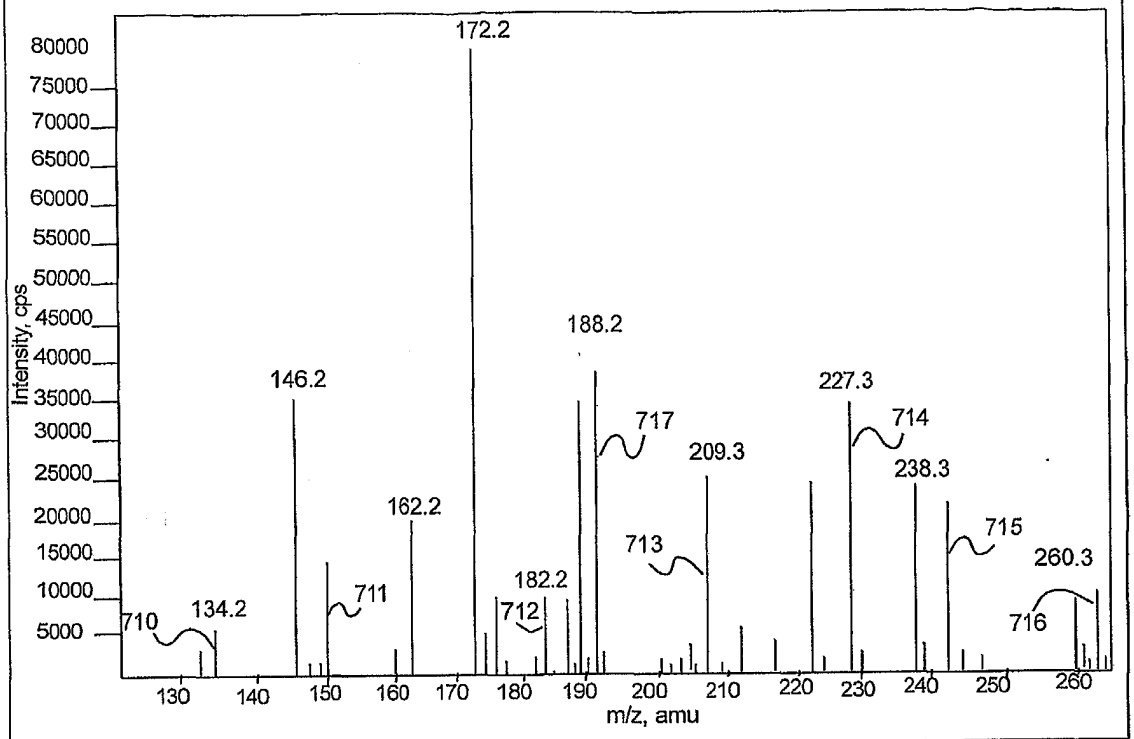


Name	Result	Concentration	
C3	-	0.88	
C4	-	0.19	
C5:1	-	0.04	
C5	-	0.13	
C4OH	-	0.11	610
(cont.)			
C16:1	-	0.18	
C16	-	2.82	
C16OH	-	0.02	
C18:2	-	0.11	
Bad Derivative	-	0.04	614
EDTA	-	0.02	616

Test	Result	Ratio	
C8_C3	-	0.03	
C3_C16	-	0.31	
C8_C16	-	9.67e-03	612
C5DC_C16	-	8.79e-03	
C14:1_C16	-	0.05	
C4_C3	-	0.22	

Mass	Result	Intensity	
347.4	-	2240	618

FIG. 3d



Name	Result	Concentration
Gly	-	143.97
Ala	-	192.28
Val	-	81.19
Gln(Glu)	-	21.17
Leu-Ile	-	112.40
Met	-	11.83
His	-	22.41
Cit	-	7.58
Phe	-	43.10
Tyr	-	95.90
Asp	-	17.09
Glu	-	128.84
Quality control	-	0.00

701

703

Test	Result	Ratio
Phe:Tyr	-	0.45
Leu:Phe	-	2.61
Leu:Ala	-	0.58
Val:Phe	-	1.88
Met:Phe	-	0.27
Glu:Phe	-	2.99

705

Mass	Result	Intensity
227.3	-	37552

FIG. 3e

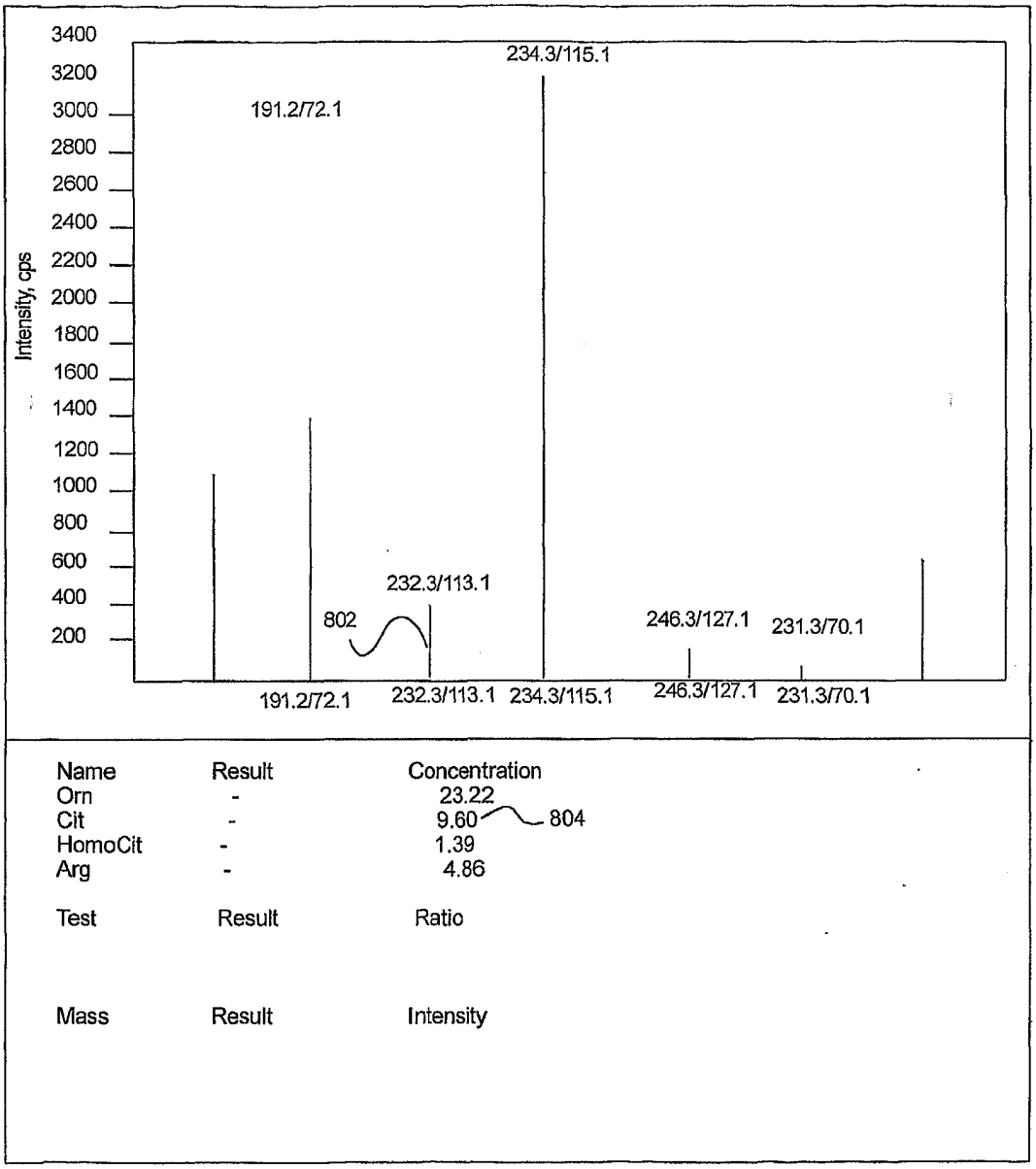

3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
Intensity, cps
234.3/115.1
191.2/72.1
232.3/113.1
802
246.3/127.1
231.3/70.1
191.2/72.1
232.3/113.1
234.3/115.1
246.3/127.1
231.3/70.1
Name Result Concentration
Orn - 23.22
Cit - 9.60
804
HomoCit - 1.39
Arg - 4.86
Test Result Ratio
Mass Result Intensity

FIG. 3f

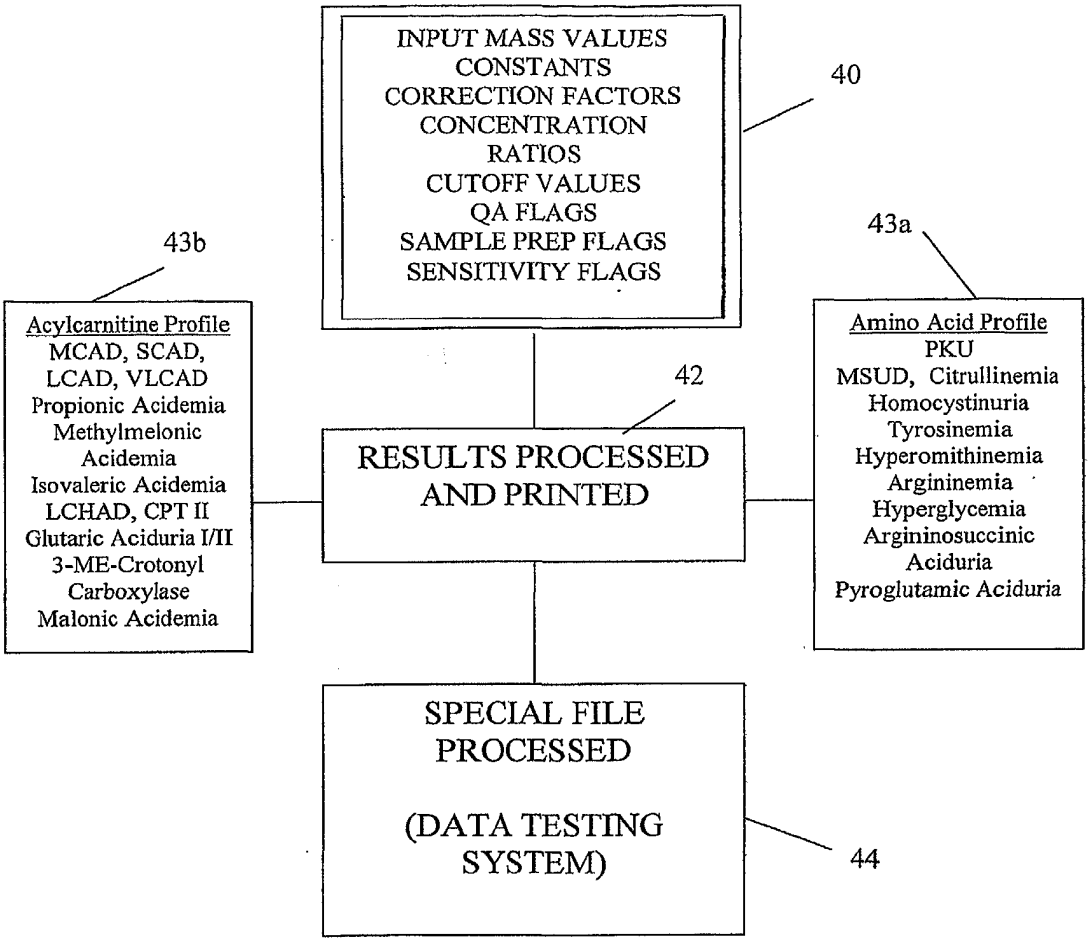

INPUT MASS VALUES
CONSTANTS
CORRECTION FACTORS
CONCENTRATION
RATIOS
CUTOFF VALUES
QA FLAGS
SAMPLE PREP FLAGS
SENSITIVITY FLAGS
40
43b
Acylcarnitine Profile
MCAD, SCAD,
LCAD, VLCAD
Propionic Acidemia
Methylmelonic
Acidemia
Isovaleric Acidemia
LCHAD, CPT II
Glutaric Aciduria I/II
3-ME-Crotonyl
Carboxylase
Malonic Acidemia
43a
Amino Acid Profile
PKU
MSUD, Citrullinemia
Homocystinuria
Tyrosinemia
Hyperomithinemia
Argininemia
Hyperglycemia
Argininosuccinic
Aciduria
Pyroglutamic Aciduria
42
RESULTS PROCESSED
AND PRINTED
SPECIAL FILE
PROCESSED
(DATA TESTING
SYSTEM)
44

FIG. 4

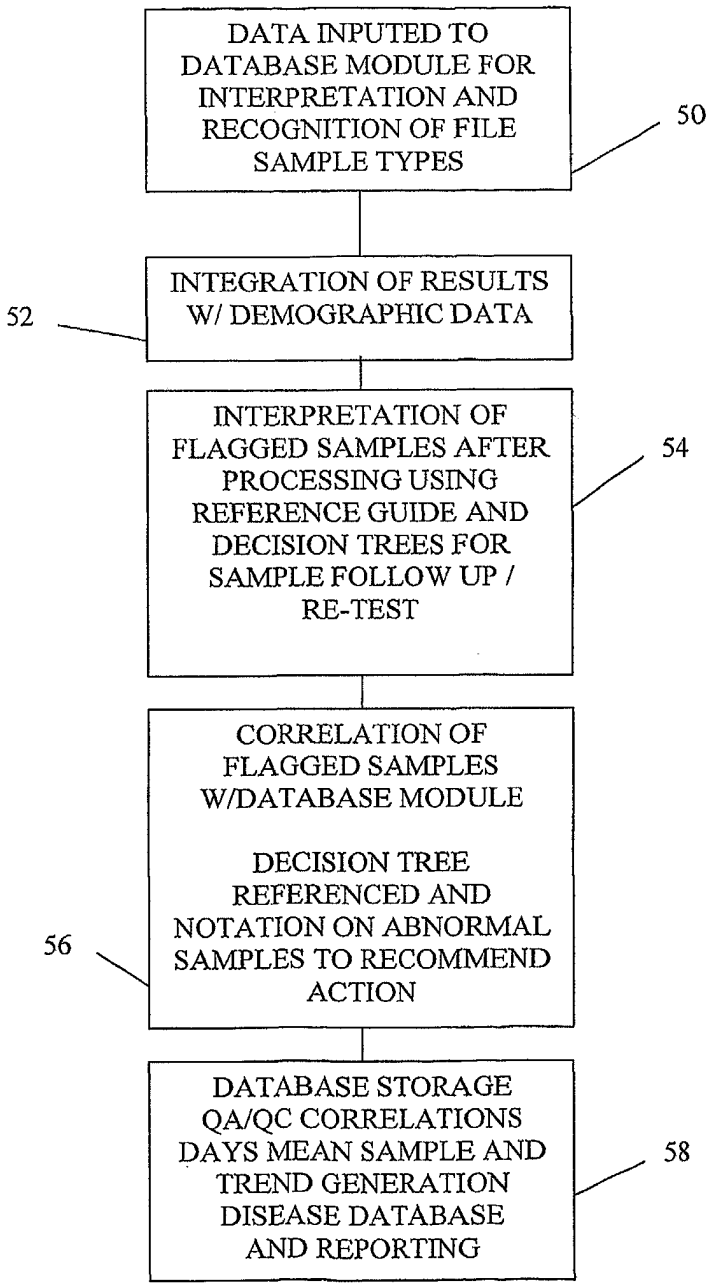

DATA INPUTED TO DATABASE MODULE FOR INTERPRETATION AND RECOGNITION OF FILE SAMPLE TYPES
50
INTEGRATION OF RESULTS W/ DEMOGRAPHIC DATA
52
INTERPRETATION OF FLAGGED SAMPLES AFTER PROCESSING USING REFERENCE GUIDE AND DECISION TREES FOR SAMPLE FOLLOW UP / RE-TEST
54
CORRELATION OF FLAGGED SAMPLES W/DATABASE MODULE
DECISION TREE REFERENCED AND NOTATION ON ABNORMAL SAMPLES TO RECOMMEND ACTION
56
DATABASE STORAGE QA/QC CORRELATIONS DAYS MEAN SAMPLE AND TREND GENERATION DISEASE DATABASE AND REPORTING
58

FIG. 5

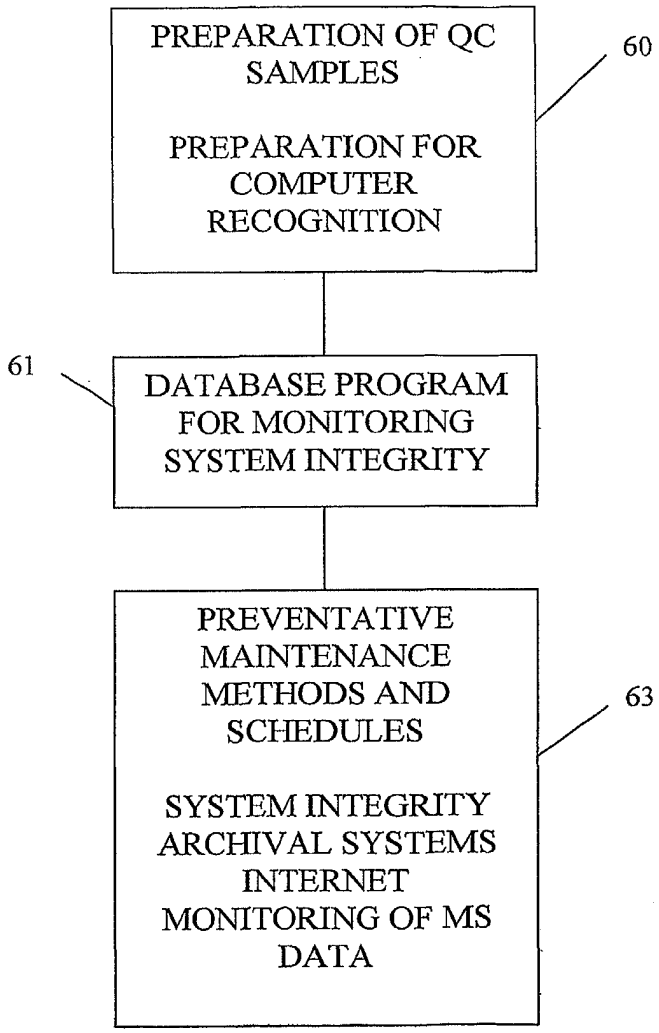

PREPARATION OF QC SAMPLES
PREPARATION FOR COMPUTER RECOGNITION
60
61
DATABASE PROGRAM FOR MONITORING SYSTEM INTEGRITY
PREVENTATIVE MAINTENANCE METHODS AND SCHEDULES
SYSTEM INTEGRITY ARCHIVAL SYSTEMS INTERNET MONITORING OF MS DATA
63

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18716

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/50, 31/00; B01D 59/44
US CL :436/8, 60, 86, 89, 90, 128, 129, 173, 174, 177, 178
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/8, 60, 86, 89, 90, 128, 129, 173, 174, 177, 178

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	M. S. RASHED et al. "Screening Blood Spots for Inborn Errors of Metabolism by Electrospray Tandem Mass Spectrometry with a Microplate Batch Process and a Computer Algorithm for Automated Flagging of Abnormal Profiles" Clinical Chemistry, July 1997, Vol. 43, No. 7, pages 1129-1141, see entire document.	27-29 --- 1-26,30-33
Y	C. G. COSTA et al, "Quantitative Analysis of Plasma Acylcarnitines Using Gas Chromatography Chemical Ionization Mass Fragmentometry" Journal of Lipid Research, January 1997, Vol. 38, pages 173-182, see entire document.	1-33

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 OCTOBER 2000	Date of mailing of the international search report 20 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ARLEN SODERQUIST DEBORAH THOMAS PARALEGAL SPECIALIST Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18716

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Y. SHIGEMATSU et al, "Modifications in Electrospray Tandem Mass Spectrometry for a Neonatal-Screening Pilot Study in Japan" Journal of Chromatography B, 1999, Vol. 731, pages 97-103, see entire document.	1-33
Y	A. W. JOHNSON et al, "The Use of Automated Electrospray Ionization Tandem MS for the Diagnosis of Inborn Errors of Metabolism From Dried Blood Spots" Biochemical Society Transactions, August 1996, Vol. 24, No. 3, pages 932-938, see entire document.	1-33
Y	D. H. CHACE et al, "Rapid Diagnosis of MCAD Deficiency: Quantitative Analysis of Octanoylcarnitine and Other Acylcarnitines in Newborn Blood Spots by Tandem Mass Spectrometry" Clinical Chemistry, November 1997, Vol. 43, No. 11, pages 2106-2113, see entire document.	1-33
Y	D. H. CHACE et al, "Rapid Diagnosis of Maple Syrup Urine Disease in Blood Spots from Newborns by Tandem Mass Spectrometry" Clinical Chemistry, January 1995, Vol. 41, No. 1, pages 62-68, see entire document.	1-33
Y	D. H. CHACE et al, "Rapid Diagnosis of Homocystinuria and other Hypermethioninemias from Newborns' Blood Spots by Tandem Mass Spectrometry" Clinical Chemistry, March 1996, Vol. 42, No. 3, pages 349-355, see entire document.	1-33
Y	D. H. CHACE et al, "Rapid Diagnosis of Phenylketonuria by Quantitative Analysis for Phenylalanine and Tyrosine in Neonatal Blood Spots by Tandem Mass Spectrometry" Clinical Chemistry, January 1993, Vol. 39, No. 1, pages 66-71, see entire document.	1-33
Y	J. L. K. VAN HOVE et al, "Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency: Diagnosis by Acylcarnitine Analysis in Blood" The American Journal of Human Genetics, May 1993, Vol. 52, No. 5, pages 958-966, see entire document.	1-33
Y	P. T. CLAYTON et al, "Screening for Medium Chain Acyl-CoA Dehydrogenase Deficiency Using Electrospray Ionisation Tandem Mass Spectrometry" Archives of Disease in Childhood, 1998, Vol. 79, pages 109-115, see entire document.	1-33
Y	D. S. MILLINGTON et al, "The Analysis of Diagnostic Markers of Genetic Disorders in Human Blood And Urine Using Tandem Mass Spectrometry with Liquid Secondary Ion Mass Spectrometry" International Journal of Mass Spectrometry and Ion Processes, 1991, Vol. 111, pages 211-228, see entire document.	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18716

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	K. HEINIG et al, "Determination of Carnitine and Acylcarnitines in Biological Samples by Capillary Electrophoresis-Mass Spectrometry" Journal of Chromatography B, 1999, Vol. 735, pages 171-188, see entire document.	1-33
Y	B. M. KELLY et al, "Electrospray Mass Spectra of Medium-Chain and Long-Chain Acylcarnitines" Organic Mass Spectrometry, 1992, Vol. 27, pages 924-926.	1-33
Y	M. S. RASHED et al, "Inborn Errors of Metabolism Diagnosed in Sudden Death Cases by Acylcarnitine Analysis of Postmortem Bile" Clinical Chemistry, August 1995, Vol. 41, No. 8, pages 1109-1114, see entire document.	1-33
Y	J. A. MONTGOMERY et al, "Measurement of Urinary Free and Acylcarnitines: Quantitative Acylcarnitine Profiling in Normal Humans and in Several Patients with Metabolic Errors" Analytical Biochemistry, January 1989, Vol. 176, No. 1, pages 85-95, see entire document.	1-33
Y	D. S. MILLINGTON et al, "Application of Fast Atom Bombardment with Tandem Mass Spectrometry and Liquid Chromatography/Mass Spectrometry to the Analysis of Acylcarnitines in Human Urine, Blood, and Tissue" Analytical Biochemistry, July 1989, Vol. 180, No. 1, pages 331-339, see entire document.	1-33
Y	Chemical Abstracts, Vol, 128, No. 19, 11 May 1998, page 323, Col. 1, abstract no. 228108p, F. INOUE et al, "Analysis of Dried Blood Spots by Electrospray Mass Spectrometry" Bull. Kyoto Univ. Educ., Ser. B, 1997, 91, 15-22, see entire document.	1-33
Y	Chemical Abstract, Vol. 123, No. 19, 6 November 1995, page 508, Col. 1, abstract no. 250402y, M. S. RASHED et al, "Diagnosis of Inborn Errors of Metabolism from Blood Spots by Acylcarnitines and Amino Acids Profiling Using Automated Electrospray Tandem Mass Spectrometry" Pediatr. Res. 1995, 38(3), 324-331, see entire document.	1-33
Y	Chemical Abstracts, Vol. 124, No. 5, 29 January 1996, page 608, Col. 2, abstract no. 49898s, N. TERADA et al, "Amino Acids and Acylcarnitines Analysis by ESIMS/MS" Nippon Iyo Masu Supekutoru Gakkai Koenshu 1995, 20, 39-44, see entire document.	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18716

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Abstract, Vol. 120, No. 13, 28 March 1994, page 536, Col. 2, abstract no. 157900n, M. S. RASHED et al, "Electrospray Tandem Mass Spectrometry in the Diagnosis of Organic Acidemas" Rapid Commun. Mass Spectrom. 1994, 8(1), 129-133, see entire document.	1-33
Y	US 4,224,031 A (MEE et al) 23 September 1980, see entire document.	1-33
Y	E. W. NAYLOR et al, "Automated Tandem Mass Spectrometry for Mass Newborn Screening for Disorders in Fatty Acid, Organic Acid, and Amino Acid Metabolism" Journal of Child Neurology, November 1999, Vol. 14, Suppl. 1, pages S4-S8, see entire document.	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18716

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN searches in CA and REGISTRY files
search terms: blood(3a)spot?, mass spectrom?, blood, dry? dried, acylcarnitine, carnitine, detect?, determin?, measur?, monitor?, assay?, analy?, test?, estimat?, standard, serine, propanamine, carboxy?, n,n,n trimethyl, oxy, oxo?, inner salt, mass spec?, spray, esi, electrospray, ionspray, thermospray, deuter?